7 Assessing Exposure of Pesticides to Bees

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7.1 INTRODUCTION

An essential component of an ecological risk assessment is a prediction of exposure of the organisms being assessed. This chapter outlines exposure pathways for the different pesticide delivery methods, both nonsystemic and systemic, and discusses methods used to predict pesticide exposure to honey bees and non-*Apis* bees. This chapter also provides an outline of techniques employed to measure pesticide residues in relevant matrices and discusses higher tier field study designs that are used to refine bee exposure assessments for specific products. Finally, this chapter presents perspectives regarding pesticide application technologies that can be employed to mitigate bee exposure, as well as future research needs to further refine exposure assessments for this taxa.

7.1.1 Potential Exposure to Foraging Bees

7.1.1.1 Sprayed Compounds

Honey bees can be exposed to direct spray, or through contact with the crop to which a pesticide is applied. Bees can be exposed to pesticides that drift to plants on the edges of the treated field, potentially leading to either contact or oral exposure, as well as water sources near the treated field that may contain residues either from drift or surface run-off. Pesticide drift can also reach hives directly if the hives are located in or near a treated field. When foliar applications are made directly onto flowers, oral exposure can occur through the

collection of contaminated pollen, nectar, or honeydew and/or by contact exposure if the product is directly sprayed on foraging bees or the plant parts that they can come in contact with during foraging.

7.1.1.2 Microencapsulated Compounds

Microencapsulated technology is designed to increase adhesion of the product to the plant surface or soil through the use of a sticking agent. Microencapsulation formulation technology is also used to control exposure by slowly releasing the pesticide. Bees can potentially be exposed to certain microencapsulated pesticides if the microcapsules are similar in size to pollen. Bees may inadvertently collect the microcapsules and bring them back to the hive. If the microcapsules are collected by bees and mixed into the beebread, the exposure may affect the whole colony as the pesticide will thus be fed to the larvae. Such incidents have been reported following the use of Pencap-M, a microencapsulated formulation of methyl-parathion (Mason, 1986).

7.1.1.3 Dust

Abraded dust that is contaminated with pesticide can be released from treated seed during planting operations involving pesticide-treated seed (Alix et al., 2009c). The exposure can be oral and/or contact from bees foraging on flowers upon which abraded dust falls. Bees may also be exposed if they fly through the dust or vapors released during planting operations (Forster, 2009; Pistorius et al., 2009; Alix et al., 2009c) or, may receive exposure if they forage on weeds and flowers (i.e., understory or in material that is adjacent to the target site) covered with contaminated dusts.

7.1.1.4 Compounds with Systemic Properties

Pesticides that have systemic properties will move within the plant and may be expressed in the pollen and nectar. Pollen and nectar of plants treated with systemic compounds (such as treated seed, soil applications, ground drench, or chemigation applications) may contain pesticide residues. These residues may be collected by foragers and brought back to the hive to be stored, processed, and fed to adults and larvae.

Bees may be exposed to pesticide residues that may occur in rotational crops or alternative forage (understory or adjacent areas) that may take up and express pesticide residues applied at an earlier date. Even if target crops are not attractive to bees, compounds that are persistent may represent a potential source of exposure through soil, or through residues in the nectar and pollen of the succeeding (rotational) crop or associated weeds. The presence of pesticide residues in a succeeding crop may be influenced by the type of crop, treatment pattern, the physicochemical properties, and of course the environmental fate of the compound.

Other potential routes of exposure for foraging bees include inhalation (Seiber and McChesney, 1987; Seiber et al., 1991), and consumption of aphid honeydew, guttation water (Girolami et al., 2009; Schenke et al., 2010), or chemigation water from soil treatments.

7.1.2 Potential Exposure to Non-foraging Bees (Wax)

All members of a colony may be potentially exposed to contaminants through the wax that composes the hive. Larvae are reared in cells made of beeswax, and as adults they are in constant contact with the wax while they are in the hive. After pupation, bees chew through the wax coating on the brood capping and emerge as an adult. During colony development, worker bees continuously modify the wax cell structure (e.g., converting male cells into worker cells, cleaning brood cells to stock honey and vice versa). Pesticides that are lipophilic tend to accumulate in wax (Tremolada et al., 2004) and if the beeswax contains pesticide residues, members of the colony, especially larvae, may be subject to contact exposure, depending upon the bioavailability of the pesticide (Chauzat et al., 2007).

7.1.2.1 Nurse Bees

For the first 1–3 weeks after emergence adult worker bees remain in the hive to perform many duties including, but not limited to, feeding and cleaning larvae, cleaning cells, building new cells, processing nectar and storing honey, packing pollen, and capping cells. Nurse bees may be potentially exposed to higher levels of pesticide residues by virtue of their duties. Nurse bees process pollen and nectar into beebread and honey, respectively, and also produce larval jelly. Nurse bees are the only caste/life-stage of honey bees that consume significant amounts of raw pollen, which is regurgitated and processed into beebread. Beebread is then stored in the hive until it is processed by nurse bees into brood food and fed to larvae. In addition, nurse bees can potentially be exposed to pesticides through water brought back to the hive for cooling and brood rearing. Nurse bees may also be exposed as they process nectar into honey within beeswax cells as well as through contact with wax while moving through the hive. Pesticides applied directly to the hive for *Varroa* sp. control and other pests are a direct route of exposure to nurse bees (Martel et al., 2007). Nurse bees can potentially be exposed to pesticides during all of these activities if residues are present in the hive.

7.1.2.2 Drones

Upon emergence as adults, drones receive food from worker bees or eat stored honey. As larvae, drones receive more food than worker larvae, but the composition of that food is similar (Free, 1977). Like larvae and nurse bees, drones may be exposed to pesticides through food or residues within the hive.

7.1.2.3 Queens

Larvae that are fed only royal jelly beyond 3 days after hatching develop into queens (Free, 1977). A queen may live within the hive from 6 months to several years. Therefore, the queen may be exposed to multiple pesticides and residues within the hive over a relatively long period of time. Feeding on royal jelly and contact with residues in the hive are the potential routes of contaminant exposure for queens.

7.1.2.4 Honey Bee Larvae

Honey bee larvae can be exposed to pesticides through ingestion of contaminated food including pollen, beebread, honey, and larval jelly. Larval worker bees are fed royal jelly (also referred to as worker jelly or larval jelly) for 3 days after egg hatch. Royal jelly is a glandular secretion from the hypopharyngeal glands of nurse bees, and consists of some white components (mostly lipids) and a clear secretion (Free, 1977). Honey bees exposed to some pesticides can potentially produce contaminated larval jelly (Tremolada et al., 2004) that could be fed to the queen, workers, and the larvae. From 4–6 days after egg hatch, worker larvae are fed beebread, which is largely processed pollen, but also includes some larval jelly, honey, and pollen (Free, 1977). The beebread can be contaminated if processed with contaminated pollen (Orantes Bermejo et al., 2010).

Water is brought back to the hive and used to cool the hive, dilute stored honey, and prepare larval food. If pesticide residues are present in this water that is brought back to the hive, larvae may be exposed through direct contact to the water or through ingestion of food prepared with the water. Larvae may also be exposed via contact exposure to pesticides that accumulate in wax or from residues on foraging bees. Additionally, larvae, as well as adults, may be exposed to insecticides/miticides applied directly to the hive by the beekeeper for *Varroa* control and/or fungicides, bactericides, or any other active substance applied for disease control.

7.1.3 Residue Movement Through the Hive

Pesticides can be transferred into the hive environment from foraging honey bees that bring residues back to the hive in contaminated pollen and nectar. Pesticide residues can also move throughout the hive as workers

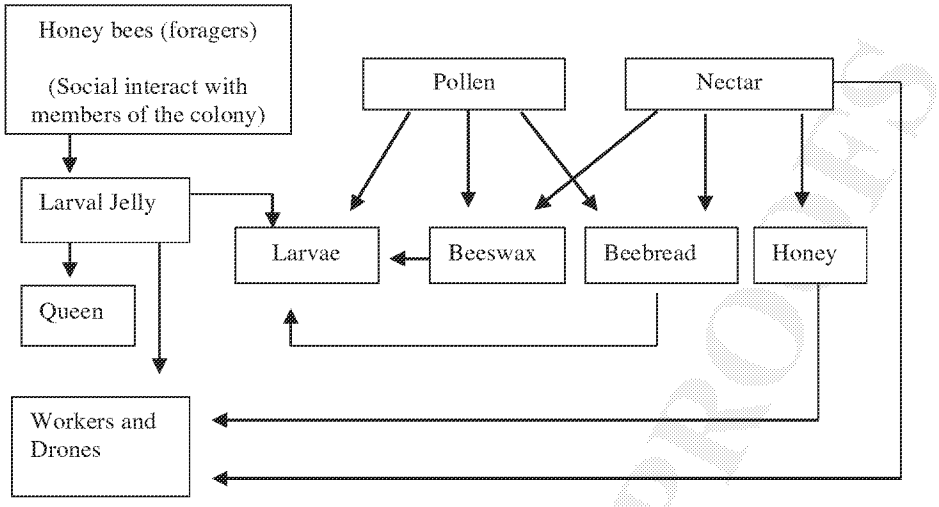

FIGURE 7.1 Conceptual model showing how contaminants may potentially reach various matrices within honey bee colonies. Pollen and nectar are the main sources of in-hive contamination. Arrows show potential major contamination transfer routes. For minor routes, please refer to the text.

pass food (especially nectar and diluted honey) among themselves as it is processed, stored, or consumed. All potential pesticide transfer to, and movement within in a hive is highly dependent on the use pattern of the pesticide product, as well as the physical and chemical properties of the contaminants. Some chemicals may persist in the hive, resulting in prolonged exposures, while others dissipate and/or degrade into metabolites. Some pesticide metabolites can also be toxic to honey bees (Suchail et al., 1999; Martel and Lair, 2011). Therefore, while research continues to shed light on the fate and movement of a compound in a hive, it is important to understand and consider these properties of a compound in assessing potential exposure. Figure 7.1 shows a conceptual model of exposure routes for pesticides to honey bee colonies.

7.2 POTENTIAL ROUTES OF EXPOSURE FOR NON-*APIS* BEES

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Most routes of exposure that have been examined for honey bees are valid for non-*Apis* bees as well. However, because of their diverse and often different biology, non-*Apis* bees may be prone to other routes of pesticide exposure. Understanding different exposure routes is important because it is not feasible to conduct tests on the more than 20 000 species of non-*Apis* bees worldwide (Michener, 2007) A risk assessment for non-*Apis* bees can be based mainly on the exposure routes reviewed for honey bees and tailored for different non-*Apis* species groups. If more specific exposure information is required for risk assessment refinements, actual measures of unique exposure pathways may be adapted from tests conducted on some key non-*Apis* species (see Section 7.11). Because of the large diversity of non-*Apis* biological features, this section will be structured around some broad features of non-*Apis* bee ecology.

7.2.1 Nesting Sites and Nesting Materials for Non-*Apis* Species

Social non-*Apis* bees, such as stingless bees nest in cavities that are usually located aboveground. In addition, plant resins used for nest construction may be contaminated by pesticide applications (Romaniuk et al.,

FIGURE 7.2 Leafcutter bee on blanket flower, photo by Mace Vaughan (Xerces Society for Invertebrate Conservation). (For a color version, see the color plate section.)

FIGURE 7.3 Micropipetting nectar samples, photo by Mike Beevers. (For a color version, see the color plate section.)

FIGURE 7.4 Hand collecting pollen by removing flower anthers, photo by Mike Beevers. (For a color version, see the color plate section.)

2003), and while honey bees also use resin in nest construction, certain non-*Apis* species employ resins to a greater extent in nest building (Murphy and Breed, 2008; Roubik, 1989). Most bumble bee species (e.g., *Bombus terrestris*, *Bombus lapidaries,* and *Bombus subterraneus*), nest underground in abandoned nests of rodents and, therefore, are protected from direct spray applications. However, other non-*Apis* species nest above ground in cavities (e.g., *Melipona* spp. and *Trigona* spp.) or under patches of grasses and vines (e.g., *Bombus pascuorum* and *Bombus ruderarius)* where there is greater potential exposure to drift, or

FIGURE 7.5 Honey bee semi-field study with *Phacelia*, photo provided by BASF SE. (For a color version, see the color plate section.)

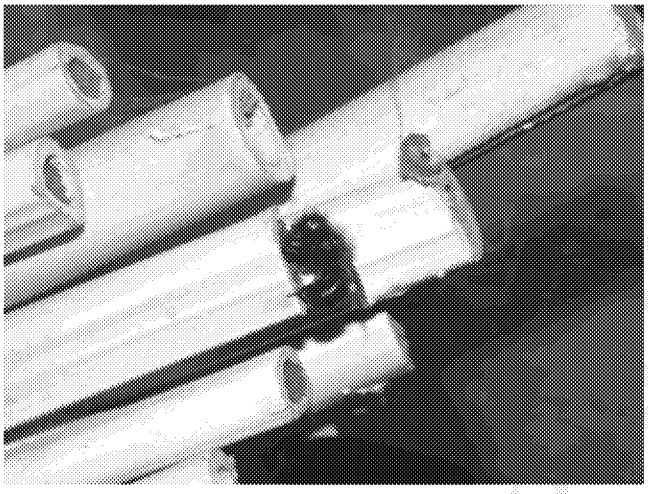

FIGURE 7.6 Mason bee, photo by Mace Vaughan (Xerces Society for Invertebrate Conservation). (For a color version, see the color plate section.)

direct pesticide applications (Pouvreau, 1984; Thompson, 2001). Stingless bees and bumble bees mainly use wax to build their nests, but, unlike honey bees, they also commonly mix it with pieces of grass, leaves, and various substrates (Pouvreau, 1984; Roubik, 1989), that may also be a source of exposure to contaminants.

Among solitary bees, the location of the nests as well as the material used to build them can vary considerably. The gregarious ground-nesting species can occur in large aggregations of several thousand individuals in natural sites (e.g., Potts and Willmer, 1998) or in man-made bee beds such as for *Nomia melanderi* (Cane, 2008). In addition, ground-nesting bees can be found along the border of fields planted with annual crops, but also in the soil within such fields (Vaissière et al., 1985; Shuler et al., 2005; Kim et al., 2006). Therefore, the dissipation rate of pesticides in soil is a key factor affecting potential exposure to species that nest in the field. Among the "tunnel nesters," leafcutter bees (Megachilidae, especially *Megachile* spp.) use excised leaf or petal pieces, as their common name suggests, to line their burrows and seal each cell once their egg has been laid on a ball of pollen and nectar. These leaf pieces are collected from a large array of plants, such as alfalfa and rose bushes.

The second largest group of solitary bees consists of species that nest in pre-existing cavities (mostly tunnels) in dead wood, hollow twigs and bamboo, or pithy stems such as elderberry (*Sambucus* spp.). These include most bees in the genera *Osmia* and *Megachile* (Cane et al., 2007). Other species, such as carpenter bees (*Ceratina* spp., *Lithurgus* spp., and *Xylocopa* spp.) drill their nest tunnels in soft wood or the soft pith of some plant stems.

Other bees build their nests with flower petals (e.g., *Hoplitis* spp.), or plant hairs (e.g., wool-carder bees such as *Anthidium manucatum*) (Gibbs and Sheffield, 2009), and many mason bees, *Osmia* spp., use mud to build partitions between the different cells of their nests (e.g., Bosch and Kemp, 2001; Mader et al., 2010), and exposure to pesticides may occur from these materials if contaminated (Waller, 1969; Johansen and Mayer, 1990). The increasing use of systemic insecticides, not only in commercial agriculture but also in residential or recreational scenarios, may result in exposure of certain species (Vera Krischik, personal communication),

especially some species of *Osmia* that chew up pieces of leaves to create walls of pulp to separate brood cells. This, however, requires further study to better understand.

7.2.1.1 Exposure at Immature Stages of Non-*Apis* Species

As stated previously, honey bee worker and drone larvae feed on food that has been processed, which may result in modifications (e.g., degradation) of pesticide active ingredients in food stores. However, this differs from scenarios of solitary non-*Apis* bees whose larvae feed directly on raw pollen and nectar in either a mass provisioning manner or sequential mass provisioning manner (i.e., brood cells are provisioned over various timeframes). As such, exposure via food may differ between *Apis* and non-*Apis* species feeding on mostly unprocessed pollen, nectar, and other floral resources (O'Toole and Raw, 1999; Pereboom, 2000). Therefore, exposure estimates based on stored honey bee pollen which is converted to royal jelly may not be predictive of the chemical residues fed to the non-*Apis* bee brood (Konrad et al., 2008). In addition, with bees that mass provision their cells (i.e., most non-*Apis* bees), the eggs and larvae are in direct contact with the pollen and nectar provision during the early life stages (i.e., the egg and first instar). Honey bees, on the other hand, are isolated in their cells and are fed progressively by nurse honey bees, and therefore, have a very different exposure profile (Winston, 1987).

7.2.1.2 Foraging Time and Mating

Among solitary non-*Apis* bees, males are the first ones to emerge from the nest, followed a few days later by females. Non-*Apis* bees vary considerably in adult size from a few mm (e.g., *Perdita* spp.) to the very large carpenter bees (*Xylocopa* spp.), and bumble bee queens (*Bombus* spp.) that routinely reach 3 cm long or more (Michener, 2007). Most non-*Apis* bees are smaller than honey bees and, therefore, can be exposed to relatively higher amounts of pesticides by contact because of the higher surface area to volume ratio of smaller species (This has been demonstrated with intraspecific (pesticide toxicity) tests that have indicated that some smaller bees are more sensitive than larger bees at similar exposures on a unit/bee basis.) (Thompson and Hunt, 1999; Malone et al., 2000).

Peak foraging time for honey bees is generally during warm, non-overcast conditions (Johansen and Mayer, 1990; Tew, 1997). However, this is not the case for many non-*Apis* bee species, such as bumble bees and mason bees (*Osmia* spp.), which are known to forage during cool, inclement weather, as well as earlier and later in the day and earlier and later in the season than honey bees (Thompson and Hunt, 1999; Vicens and Bosch, 2000; Bosch and Kemp, 2001; Thompson, 2001). Similarly, squash bees (*Peponapis*, and *Xenoglossa* spp.) are active in the early predawn hours (Sampson et al., 2007). In addition, males of many non-*Apis* bees often spend the night in flowers or hanging from plants, potentially leading to higher exposures (Sapir et al., 2005). However, male squash bees that spend the night in closed squash blossoms may receive some level of protection from night time pesticide applications because the blossoms close tightly around them.

7.2.1.3 Food Sources

Honey bees are extreme generalists in that a colony will forage for nectar and pollen on a large array of plant species (polylecty). This is not so for most non-*Apis* bees, especially for the 80% or more which are solitary. These species often gather their pollen on a few species of taxonomically related plant species (oligolecty) and sometimes on a single species. Indeed, non-*Apis* bees may also forage, and even specialize, on plants not readily visited by honey bees (e.g., potato, many legumes, and some ornamentals). As a result,

pesticide exposure (to generalists) may be "diluted" from various floral resources across a wide landscape. For example, tomato and potato flowers do not produce nectar but will release their pollen through buzz pollination (sonication). Although, it is possible that pollen from flowers of this type could be shielded from foliar pesticide applications (because of the unique plant morphology), and considered safe for honey bees, they remain a potential exposure scenario for non-*Apis* bees.

7.2.1.4 Size

Another factor affecting foraging and exposure in non-*Apis* bees is the size of some non-*Apis* bees, and the relationship between foraging distance and species size. Some non-*Apis* bees are much smaller than honey bees (e.g., bees of the genera *Perdita* or *Dialictus* in the United States and *Nomioides* in Europe), and therefore are subject to relatively greater exposure because of the higher surface area to volume ratio of smaller bodies (i.e., μg of pesticide that contacts the body/mg body weight). Indeed, even intraspecific tests of pesticide toxicity to bumble bees have confirmed that smaller bees may be more effected than larger bees for a specific dose (Van der Steen, 1994; Thompson and Hunt, 1999; Malone et al., 2000).

A second size-related factor affecting potential exposure of non-*Apis* bees is the relationship between size and foraging distance. Whereas large bees, such as honey bees, bumble bees, or carpenter bees (*Xylocopa* spp.) easily forage over several kilometers from their nest (Beekman and Ratnieks, 2000; Goulson and Stout, 2001; Pasquet et al., 2008); small bees may only fly a few hundred meters from their nest site (Greenleaf et al., 2007). This factor may potentially result in higher exposure to small bees, compared to larger species, that are attracted to blooming crops, where their limited foraging range necessitates nearby nesting, and ongoing exposure to pesticide applications throughout the growing season. In some landscapes (e.g., New Jersey, USA), small bees (e.g., *Halictus* and *Lasioglossum* spp.) perform a significant amount of crop pollination (Winfree et al., 2007, 2008).

Somewhat related to foraging distance is the tendency of certain solitary bees to collect pollen from one area, and often from only one or a few plant species, whereas honey bees forage on a wide variety of plant species across a large landscape. Honey bee foraging areas and sources of nectar and pollen can vary considerably from one day to the next (Visscher and Seeley, 1982). Therefore, due to the foraging behavior, the pesticide residues on one crop may be diluted in a honey bee colony diet, but not so in the nest of a non-*Apis* species.

7.3 METHODS AND MODELS FOR ESTIMATING EXPOSURE OF BEES TO PESTICIDES

Currently, there are no globally accepted approaches for estimating exposure of pesticides to bees for screening-level risk assessments. Participants of the Workshop reviewed current methodologies employed in the United States and European Union, and evaluated information that can be used or developed to establish exposure estimates for screening-level risk assessments for both honey bees and non-*Apis* bees.

7.3.1 Screening Level Exposure Estimates

Atkins et al. (1981) conducted laboratory contact toxicity studies and corresponding field studies with 65 pesticides. The field hazards were studied in a large number of commercial fields during bloom using crops that were highly attractive to honey bees. Data developed by Atkins et al. (1981) indicated that, for foliar-applied products, the median lethal dose (LD50) as measured in micrograms of active ingredient per bee (μg a.i./bee) can be coverted and expressed as the equivalent number of kilograms of chemical per hectare (kg a.i./ha) (that would yield an LD50) by multiplying by 1.12. For example, an acute contact LD50 of 1 μg

a.i./bee (highly toxic according to Atkins et al. (1981) classification scheme) would equate to an application rate of 1.12 kg a.i./ha, (or pound per acre). In the European Union, the hazard quotient (HQ) approach is used as a screening-level assessment to distinguish between compounds with either potentially low or high risk of acute poisoning from foliar pesticide applications. The HQ relates the application rate of a product with laboratory oral and contact LD50 values.

$$HQ = \text{Application rate (g a.i./ha)/Contact or Oral LD50 (}\mu\text{g a.i./bee)}^{1}$$

7.3.1.1 Environmental Protection Agency Residue Unit Dose (TREX), Comparison of Lab Contact Toxicity Data with Residue Data From TREX

Environmental Protection Agency (EPA) has typically employed the terrestrial residue exposure model (TREX) when investigating foliar-applied pesticides. This model is used to predict residues on food items (e.g., vegetation, seeds, insects) for birds and mammals, and is based on a nomogram developed by Hoeger and Kenaga (1972). The contact exposure to a bee (which to this point has only been done for endangered species analysis) is calculated by multiplying the residue predicted for broadleaf plants/small insects by the assumed weight of a foraging honey bee (0.128 g) (Mayer and Johansen, 1990) to establish a dose per bee (μg a.i./bee).

Although the TREX method could potentially be useful for developing a screening-level exposure estimate for bees in a risk assessment process, the values developed by Hoeger and Kenaga (1972) are not based on residue data for insects but rather on plants or plant parts of similar size (Fletcher et al., 1994). Data from Hart and Thompson (2001) indicate that the 95th percentile value for an insect residue per unit dose (RUD) is 24 mg/kg compared to 135 mg/kg for broadleaf plants (EPA's surrogate for small insects) which is approximately six-fold higher. Data from additional studies (Brewer et al., 1997; Fischer and Bowers, 1997) also suggest that the insect residue estimates developed by Hoeger and Kenaga (1972) are greatly overestimated.

7.3.1.2 ICPBR (EPPO) Proposal for Seed Treatment or Soil-Applied Systemic Compounds

The main route of exposure of bees to residues from systemic compounds (such as those applied as a seed treatment or soil application) is through the translocation of the compound into nectar and pollen. Data on measured residue levels in different plant parts have been compiled and analyzed by Alix et al. (2009a). Residue levels in plant parts were measured after treatment with systemic insecticides for the purpose of developing Tier 1 exposure assessments. The compiled residue database considered residue values as close as possible to flowering. Based on their analysis, a default maximum residue value of 1 mg a.i./kg plant matrix has been proposed as a peak value for the screening-level exposure estimate for systemic compounds used as seed treatments or applied to soil (Alix et al., 2009a; Alix and Lewis, 2010). In the event the Tier 1 risk assessment based on this worst-case estimate indicates a potential risk, actual measured residues from higher tier studies can be used for a refined risk assessment. If there is a need to transform the Tier 1 predicted concentrations in pollen and nectar into predicted doses for honey bees, it is recommended to follow the proposals as outlined by International Commission for Plant–Bee Relationships (ICPBR) (Alix et al., 2009a), which uses pollen and nectar consumption rates by different castes of honey bees (Rortais et al., 2005). The published consumption rates are provided later in this chapter (see Section 7.7).

[1] See Chapter 8 for a discussion on acute (dermal or oral) toxicity tests.

7.4 PHYSICAL AND CHEMICAL PROPERTIES OF PESTICIDE ACTIVE INGREDIENTS WHICH AFFECT EXPOSURE

The physicochemical properties of the pesticide active ingredient determine its fate in soil and in hive matrices which can affect the exposure of the various life stages of both *Apis* and non-*Apis* species to these chemicals.

1. Fate in soil—systemic products
   Systemic products applied to soil can be taken up by the plant and translocated into plant foliage, floral nectar, and pollen. Persistent systemic products that remain in the soil for over an year could potentially be translocated into the nectar and pollen of rotational crops planted in succeeding years. The dissipation time (DT50) is used to characterize the persistence of pesticides in soil.

   Physicochemical properties of the pesticide active ingredient that can affect persistence in soil include water solubility, the octanol–water partition coefficient (K_{ow}), dissociation constant (K_a), the soil adsorption coefficient (K_d), and the organic carbon partition coefficient (K_{oc}). Pesticides with high water solubility and low K_{oc} (e.g., <50) values have a higher potential for mobility, do not strongly adsorb to soil particles and can be prone to leaching depending on soil conditions, weather, and persistence of the compound. The log of the K_{ow} (log K_{ow} or log P) is the measure of a chemical's propensity to bioaccumulate. Pesticides with a high log P (e.g., >3) usually have low water solubility and are not highly mobile in soil. The log of the dissociation constant (pK_a) is a measure of the extent to which a substance ionizes in equilibrium with water. The p*K*a of a pesticide indicates the ratio of the forms (ionized or undissociated) in which the chemical will exist in environments of various pH values, and the extent of its potential involvement in ion-exchange binding processes in soils or sediments. The form of a pesticide (anion or cation) can influence its mobility and hence persistence in soil. Soil type and meteorology (amount of rainfall, temperature) can also influence the persistence of a pesticide in soil.

   Specific criteria to classify compounds as being persistent in soil have been identified by the European Union (EEC, 2006) and other regulatory agencies to trigger the requirement of rotational crop residue studies (used to inform human health risk assessment). It has been proposed that similar criteria be used to require assessment for the risk of residues in pollen and nectar for succeeding crops (Alix and Lewis, 2010).

Author: Please provide complete details for the reference citation "EEC, 2006" to be included in the reference list

2. Fate in hive matrices—systemic and nonsystemic products
   Physicochemical properties including water solubility, log P, and the pK_a can influence the fate of the active ingredient in the hive. Compounds with a high log P that are hydrophobic (i.e., tending to be insoluble in water) may accumulate in wax, pollen, and beebread, which contain lipids. Compounds with a high solubility in water (hydrophilic) can partition to nectar and honey which contain water. If the compound dissociates, the dissociation constant may be used to indicate the fate in acidic matrices such as honey.

7.5 INFORMATION NEEDED TO DEVELOP REFINED PREDICTIVE EXPOSURE MODELS

As stated earlier, there are no defined predictive models currently used for estimating the exposure levels in bees or bee matrices for use in a screening-level ecological risk assessment. The procedures described here that have been previously used by the European Union and Canada for example, and employ values for potential exposure, have been effective in screening-out compounds that have low potential risk to adult worker bees from foliar-applied products. However, for crop protection products where potential risk cannot

be excluded based on current Tier 1 screening analysis, the current method to refine assessments consists of higher tier effects or exposure assessment studies (e.g., EPA Tier 2 foliar residue study, EPPO tunnel test).

Optimally, there should be methods to predict residue levels in relevant matrices (e.g., bees, pollen, nectar). These predicted exposure concentrations could then be used to compare with laboratory toxicity data, such as acute contact LD50 values for adult bees, and acute and chronic dietary toxicity data for adult bees and larvae to estimate risk to both foraging bees and other castes and life-stages in the hive, including larvae.

7.6 PREDICTED CONTACT EXPOSURE FOR FOLIAR-APPLIED PRODUCTS

For foliar-applied products, the prediction of residues on foraging bees due to contact exposure (i.e., direct spray on foraging bees or bees contacting residues post spray) can be estimated. The US EPA has proposed using predicted concentrations in insects based on estimates in their TREX wildlife exposure model. However, as noted earlier, there are some inherent uncertainties with using this approach. In this approach, values from TREX Version 1.4.1, which relies on residue estimations developed by Hoeger and Kenaga (1972) for plants, fruits, and seeds, would be used as surrogate data to estimate contact exposure for insects. However, actual field residue data are available for honey bees (Koch and Weißer, 1997) and a variety of flying, soil-dwelling, and leaf-dwelling arthropods (Schabacker et al., 2005) that can be used for estimating contact exposure to bees. In a multiyear study by Koch and Weißer (1997), the fluorescent tracer sodium fluorescein was applied to flowering apple orchards or flowering *Phacelia* fields while honey bees were actively foraging, to determine contact doses in individual honey bees. After applications of 20 g sodium fluorescein/ha, doses in honey bees ranged from 1.62 to 20.84 ng/bee, and 6.34 to 35.77 ng/bee for honey bees foraging in apples and *Phacelia*, respectively. If the maximum detected residue in this study (35.77 ng/bee after an application of 20 g/ha) was used as a point estimate for a screening-level exposure assessment, a predicted environmental dose due to contact exposure (PEDc) in adult honey bees after an application of 1 kg/ha (1000 g/ha) would be 1789 ng/bee or 1.79 μg/bee. The assumption here is that there will be a linear relationship between application rate and contact dose of foraging bees, which is an area of uncertainty.

In the report by Schabacker et al. (2005), maximum residues in flying, ground-dwelling, and foliage-dwelling arthropods from a number of field trials were compiled and residue unit doses (RUDs) were calculated. The mean and 90th percentile RUDs in mg/kg after application of pesticides at a rate of 1 kg a.s./ha are summarized in Table 7.1.

When residue data for flying insects are used to develop a screening-level point estimate for contact exposure of foraging bees, a 90th percentile PEDc after an application of 1 kg a.i./ha is calculated to be 0.84 μg/bee. This is derived by multiplying the 90th percentile concentration in flying insects (6.6 mg/kg) by the weight of an adult foraging honey bee (128 mg) (Mayer and Johansen, 1990). This point estimate (0.84 μg/bee) is close to the exposure value calculated using the data of Koch and Weißer (1.79 μg/bee), and is consistent with the data developed by Atkins et al. (1981), where a dose of 1 μg/bee represents an application rate of 1 lb a.i./acre. Therefore, according to the Atkins method, an application of 1 kg a.i./ha is equivalent to an exposure value of 0.89 μg/bee. Based on this information, a worst-case estimate PEDc to honey bees after an application of 1 kg a.i./ha is 1.79 μg/bee.

To evaluate the sensitivity of the proposed point estimate of exposure for honey bees, a generic data set (LD50 values) can be used to calculate HQs and toxicity/exposure ratio (TERs),[2] along with the value of 1.79 μg/bee after an application of 1 kg a.i./ha. Using a generic data set with an application rate of 100 g a.i./ha, the corresponding HQ, TER, and RQ values are summarized in Table 7.2.

[2] TER = LD50 in μg a.i./bee/PEDc in μg a.i./bee; and, Risk Quotients (RQ) = PEDc/LD50.

TABLE 7.1
Predicted Concentrations (in mg/kg) After Foliar Application of 1 kg/ha

Arthropod Classification	Mean Predicted Concentration (mg/kg)	90th Percentile Predicted Concentration (mg/kg)
Flying insects	1.4	6.6
Ground dwellers (orchard/vines, grasslands, late growth stages of leafy crops and cereals (insecticides and fungicides))	3.6	9.8
Ground dwellers (orchard/vines (herbicides), early growth stages of leafy crops and cereals (all pesticides)	6.7	15.6
Leaf dwellers	9.5	47.8

Source: Data from Schabacker et al. (2005).

According to Annex VI of the EU Uniform Principles, a TER of ≥10, designed to cover potential variabilities (such as interspecies), typically indicates acceptable risk for terrestrial organisms, and has been recommended as an appropriate assessment factor for oral exposure to systemic insecticides by ICPBR (Alix et al., 2009a, 2009b; Alix and Lewis, 2010). US EPA on the other hand uses a level of concern (LOC) RQ of 0.1 for non-listed threatened or endangered aquatic or avian species. Based on this analysis, the screening-level risk assessment based on a PEDc of 0.179 μg/bee is in line with the current European Union screening HQ of 50.

Although the published field trial data (Koch and Weißer, 1997) for residues on honey bees are most appropriate for developing exposure estimates for honey bees, it might be more appropriate to use the data for leaf-dwelling and soil-dwelling arthropods developed by Schabacker et al. (2005) to address exposure to leaf-dwelling and soil-nesting non-*Apis* bee species, respectively. Therefore, for the initial conservative point estimate of contact exposure, the 90th percentile predicted concentration for leaf-dwelling arthropods (47.8 mg/kg) can be used to develop a PEDc for leaf-dwelling species, while the 90th percentile predicted concentration for soil-dwelling arthropods (15.6 mg/kg) can be used to develop a PEDc for soil-nesting species. However, in order to complete this analysis and develop recommended PEDc values for leaf-dwelling

TABLE 7.2
Comparison of Hazard Quotient (HQ), Toxicity/Exposure Ratios (TER) and Risk Quotients (RQ) Assuming a Predicted Contact Exposure Dose (PEDc) of 1.79 μg a.i./bee After an Application of 1 kg a.i./ha

Use Rate (kg/ha)	PEDc (μg/bee)	Contact LD50 (μg/bee)	HQ	TER	RQ
0.1	0.179	1	100	5.6	0.18
0.1	0.179	2	50	11	0.09
0.1	0.179	5	20	28	0.036
0.1	0.179	20	5	112	0.009

and soil-nesting non-*Apis* bees, focal species need to be identified. For leaf-dwelling species, the leafcutter bee (e.g., *Megachile rotundata*) is recommended as a surface dwelling non-*Apis* reference species, while the bumble bee (*Bombus* spp.), which typically nests on or underground, or the mason bee (*Osmia* spp.), which collects mud for nest construction, is recommended for soil-nesting (gregarious) focal species. Ideally, ground-nesting solitary bees, such as sweat bees (e.g., *Halictus* or *Lasioglossum* spp.), squash bees (*Peponapis* or *Xenoglossa* spp.), or alkali bees (e.g., *Nomia melanderi*) could also be considered a representative soil-nesting species, for these insects dig nests underground. However, at least in North America, only *Nomia melanderi* is currently managed successfully on a larger scale. With the identification of focal species, the typical body weights of the species can be used to convert predicted exposure concentrations in mg/kg to PEDc values in μg/bee for direct comparison to laboratory toxicity data.

Prior to adopting this proposed methodology into a formal regulatory assessment paradigm for bees, the method should be used to calculate toxicity/exposure ratios for some representative compounds to ensure that the exposure assessment methodology is sensitive enough to predict an acute risk to compounds that are highly toxic to non-*Apis* bees (e.g., pyrethroid insecticides), while not predicting a high risk for compounds that are known to have low inherent toxicity and present a low risk to non-*Apis* bees. Such an exercise would provide some feedback that the proposed methodology would not potentially be inconsistent with protection goals.

7.7 PREDICTED DIETARY EXPOSURE FOR FOLIAR-APPLIED PRODUCTS

For assessing acute or chronic dietary risk to adults or larvae, predicted concentrations in relevant food items (e.g., pollen, nectar, beebread, honey, and larval jelly) should be used as the dietary exposure estimate. Currently, models to predict residues in these items from foliar-applied pesticide products do not exist. Although the results from survey-style analysis indicate that agricultural pesticides are entering managed honey bee colonies through contaminated pollen (Chauzat et al., 2010; Mullin et al., 2010), there are limited published data from controlled studies that relate foliar application rates to measured pesticide levels in pollen and nectar or in any processed hive food.

In a study by Choudhary and Sharma (2008), residues of three foliar-applied pesticides were determined in nectar and pollen following applications to flowering mustard. Pesticides evaluated in this two-year study were endosulfan, lambda-cyhalothrin, and spiromesifen. Mean measured residues in pollen and nectar, and predicted concentrations after application of 1 kg a.i./ha are summarized in Table 7.3.

In a study by Wallner (2009), residues of the fungicides boscalid and prothioconazole were determined in pollen and nectar samples from foraging bees following applications to oilseed rape (canola). Mean measured residues in pollen and nectar and predicted concentrations after application of 1 kg a.i./ha are summarized in Table 7.4.

Finally, in a study by Dinter et al. (2009), concentrations of the insecticide chlorantraniliprole in pollen and nectar collected from foraging bees following applications to *Phacelia* in a semi-field study were determined. The maximum concentrations in pollen and nectar, 1 day after treatment are summarized in Table 7.5.

It is difficult to draw any firm conclusions based on these limited data. For instance, there is not a linear relationship between application rate and measured concentration in pollen and nectar across the different compounds. Therefore, the predicted concentrations after applications of 1 kg/ha (i.e., PEDc's) may be greatly exaggerated for some compounds. It is likely that the variation in residue levels seen between these two studies (Dinter et al., 2009 and Wallner, 2009) is a result of different factors such as sampling, extraction methods, fate properties of the different compounds, or product formulation.

Although limited published data are available for maximum residue levels in nectar and pollen after controlled applications of foliar products, there is likely to be a significant amount of data that have been developed by pesticide manufacturers for individual products. Therefore, the participants of the workshop

TABLE 7.3
Day 0 Measured Concentrations of Three Foliar Applied Pesticides in Pollen and Nectar After Application to Flowering Mustard

Compound	Application Rate (g a.i./ha)	Mean Measured Residues Nectar[a] (mg/kg)	Mean Measured Residues Pollen[a] (mg/kg)	Mean Predicted Nectar Residues (mg/kg) After Application of 1 kg/ha	Mean Predicted Pollen Residues (mg/kg) After Application of 1 kg/ha
Endosulfan	525	1.725 ± 0.031 1.583 ± 0.006	2.126 ± 0.088 2.068 ± 0.048	3.15	3.99
Lambda-cyhalothrin	75	0.858 ± 0.038 0.728 ± 0.022	1.607 ± 0.004 1.577 ± 0.018	10.6	21.2
Spiromesifen	225	1.541 ± 0.078 1.401 ± 0.016	2.003 ± 0.040 1.799 ± 0.033	6.54	8.45

Source: Data from Choudhary and Sharma (2008).
[a]Mean measured residues from two successive application and sampling years.

proposed that nectar and pollen residue data from semi-field exposure studies conducted according to EPPO guidelines be compiled and analyzed. These data should represent maximum residues in bee food items in a bee-attractive crop, and developing models around these data would likely provide realistic, worst-case predicted residues for a screening-level risk assessment.

Once these data are compiled, a conservative estimate for residues on/in pollen and nectar (e.g., 90th percentile RUDs) can be used to calculate TER or RQ values. These screening-level predicted values would represent a conservative estimate of dietary exposure for honey bees from foliar application of pesticide products. For a dietary risk assessment, the predicted concentration of residues in food items can be directly compared with the results from dietary toxicity studies with adult bees and bee larvae, if the results from

TABLE 7.4
Day 0 Measured Concentrations of Two Foliar Applied Fungicides in Pollen and Nectar Collected from Honey Bees After Application to Flowering Oilseed Rape

Compound	Application Rate (g a.i./ha)	Mean Measured Residues Nectar (mg/kg)	Mean Measured Residues Pollen (mg/kg)	Mean Predicted Nectar Residues (mg/kg) After Application of 1 kg/ha	Mean Predicted Pollen Residues (mg/kg) After Application of 1 kg/ha
Boscalid	500	1.43	26.2[a]	2.86	52.4
Prothioconazole	250	0.69	nd (LOQ = 0.001)	2.76	

Source: Data from Wallner (2009).
[a]Concentrations 1 day after treatment, which were higher than day 0 values.

TABLE 7.5
Day 1 Measured Concentrations of Chlorantraniliprole in Pollen and Nectar Collected from Honey Bees After Application to Flowering *Phacelia*

Compound	Application Rate (g a.i./ha)	Maximum Measured Residues Nectar (mg/kg)	Maximum Measured Residues Pollen (mg/kg)	Maximum Predicted Nectar Residues (mg/kg) After Application of 1 kg/ha	Maximum Predicted Pollen Residues (mg/kg) After Application of 1 kg/ha
Chlorantraniliprole	60	0.033	2.60	0.55	43.3

the studies are expressed as exposure concentrations (i.e., LC50, NOEC). However, if the toxicity results are expressed as a dose (i.e., LD50 in μg/bee), the predicted dose can be calculated based on predicted concentrations on food items and consumption rates by different castes of bees. Honey bee consumption data, based on complete life-stages, have been reported by Rortais et al. (2005), and are summarized as follows.

Nectar foragers: 224–898.8 mg sugar
Pollen foragers: 72.8–109.2 mg sugar
Nurse bees: 65 mg pollen
Worker larvae: 59.4 mg sugar + 5.4 mg pollen
Drone larvae: 98.2 mg sugar

The following daily consumption rates for the different honey bee casts were calculated by Thompson (2007):

Nectar foragers: 32–128.4 mg sugar/bee/day
Pollen foragers: 10.4–15.6 mg sugar/bee/day
Nurse bees: 6.5 mg pollen/bee/day
Worker larvae: 11.9 mg sugar + 1.1 mg pollen/bee/day
Drone larvae: 15.1 mg sugar/bee/day

For dietary exposure estimates, it will be important to choose the appropriate consumption rate with respect to life stage, that is, the daily consumption rate should be compared with acute oral toxicity data to estimate acute risks, while life-stage consumption data should be compared with chronic toxicity data to estimate chronic risk.

7.8 PREDICTED EXPOSURE FOR SOIL AND SEED TREATMENT SYSTEMIC COMPOUNDS

For soil-applied or seed treatment systemic products, the current ICPBR proposal recommends using a default maximum exposure value of **1 mg/kg for pollen and nectar**, which is based on the analysis of existing residue data (Alix et al., 2009a). Currently, the number of standardized exposure studies evaluating residues in pollen and nectar for systemic pesticides is limited to a few compounds for the same class of chemistry (i.e., neonicotinoids) (Alix et al., 2009b). Therefore, there may not be enough data to develop a

predictive exposure model applicable to all soil-applied or seed treatment systemic compounds. In the case of systemic compounds, it appears that residues in pollen and nectar are not only influenced by the physical and chemical properties of the compound (e.g., K_{oc}, soil DT50, K_d, pollen and nectar uptake and dissipation), but also by soil properties, crop, weather, and application timing versus the time of bloom. Therefore, as pollen and nectar residue data for other classes of systemic compounds are developed, the additional variables should be considered. As more residue data are developed for systemic compounds (both neonicotinic and other classes), the concept of developing a predictive screening-level exposure model should be explored further. In the interim, the default value of 1 mg/kg is recommended as the point estimate for exposure in Tier 1 risk assessment for dietary exposure to systemic compounds, as it represents a current worst-case estimate of residues in matrices that are consumed by bees (i.e., pollen and nectar). However, as more data are developed for systemic compounds, the value of 1 mg/kg should be re-evaluated to ensure that it is sufficiently conservative for use in a screening-level risk assessment.

7.9 PREDICTED EXPOSURE FOR TREE-INJECTED COMPOUNDS

Certain insecticides can be directly injected into tree trunks for control of wood boring insects. The chemical enters the xylem and is systemically transported to all parts of the tree including nectar (if produced) and pollen, and potentially propolis, which is not consumed, but is used by bees in the construction and maintenance of nests and hives. There is a scarcity of data on residues of pesticides resulting from tree injections. Until more data are developed or collected, it is unclear if the residue value of 1 mg/kg, as proposed by ICPBR for soil and seed treatments, is appropriate as a maximum default residue for a screening-level risk assessment for tree injection.

7.10 MEASURING PESTICIDES IN MATRICES RELEVANT FOR ASSESSING EXPOSURE TO BEES

When quantification of pesticide residues in bees or bee food is required to refine an exposure assessment, it must be determined whether the goal is to assess exposure of adult forager bees or other members of the hive (queen, nurse bees, drones, and larvae). To determine exposure of foragers from foliar applications, analysis of bees collected from the sprayed crop can be conducted. For exposure of forager bees from oral sources, samples of nectar and pollen can be collected by hand from flowers or from foraging bees on the crop. Bees may be sampled by drawing nectar from the honey stomach and pollen can be removed from the pollen baskets. Whether it is more time- and cost-effective to use bees to collect samples or to do it by hand sampling is dependent on the type of crop flower being sampled.

Where collection of nectar from the target crop is possible by hand, this can be done by inserting a microcapillary tube or pipette into the nectary and extracting the nectar. Collection of pollen by hand can be done by shaking flowers or using scissors to remove anthers followed by separation of the pollen from the anthers either in the field or after transportation to a laboratory. Flowers from several crops have very little, if any, nectar and pollen, making hand collection impractical. In these instances, bees can be used to collect the samples. Obtaining nectar samples using bees can be done by collecting the bees that are actively foraging on flowers in the crop of interest (such as by vacuuming, which, in certain cases may be impractical). Another way to sample bees is by collecting them at the hive entrance. In either scenario, verification of exposure from the crop of interest should be done by identifying pollen brought back to the hive or by confining the bees during the exposure portion of the study using a semi-field study design. To obtain the nectar sample from honey bees, the honey stomach can be dissected from the bee and the contents drained into a vial or be pierced with a syringe or micropipette and the nectar extracted. Pollen can be obtained from bees collected from flowers or at the hive entrance by removing the pollen from the pollen baskets. Pollen samples can also be

collected in pollen traps attached to the hive entrance. If either pollen or nectar cannot be efficiently collected in large enough quantities for residue analysis, whole flower samples could also be analyzed for possible use as a surrogate (pending further collection and analysis of these data).

Samples from the hive can be drawn for potential exposure to residues in stored pollen, nectar, and larval jelly. Stored pollen can be sampled by identifying frames where fresh pollen is being stored and removing this pollen with a spatula from individual cells. Adding an empty comb can ensure that the pollen and nectar is freshly collected. Nectar can be sampled by identifying the frame where fresh nectar is being stored, removing the frame from the hive, and shaking the frame into a large pan to release the nectar. The released nectar can then be transferred to a vial using a pipette, or pouring if the volume allows. Alternatively, fresh nectar can be identified and extracted from individual cells using a syringe or pipette and transferred to a vial. Larval jelly can be identified on the frames and collected either by extracting it from the cells with a capillary tube or pipette, or by removing the larvae and scooping out the jelly with a spatula and transferring it to a vial.

All samples collected in the field should be kept on ice until received by the analytical laboratory. At the laboratory, samples should be stored frozen (−20°C) and protected from light until analysis. Experience shows that plastic storage containers should be used with caution because some pesticides can sorb to plastic. Standardized procedures for sampling, including appropriate storage and transport, should be established in order to avoid contamination, and provide adequate sample size. Specific, statistically valid plans for sample size and number also should be established in the study protocol. Dedicated coolers, chain of custody, records of transport and storage conditions, and other appropriate good laboratory practice procedures should be used and documented to ensure sample integrity. The quantity of samples needed for analysis of pesticide residues should be determined prior to sampling and might vary based on the limits of detection and limits of quantification for each pesticide in the individual matrices. Use of spiked samples, to accompany samples collected from the field, can be used to ensure sample integrity (as well as sample stability). Analytical methods also need to be properly validated to ensure that extraction methods are adequate and the residues of interest are accurately identified.

At the present time, it is recommended that collection of nectar and pollen directly from the flowers, or collecting and removing pollen and nectar from foraging bees would be the most conservative and most relevant estimates of exposure for bees outside the hive. For larvae, nurse bees, drones, and the queen in the hive, sampling freshly deposited nectar and pollen from the combs would be the most conservative dietary exposure estimate; considering additional processing of these materials by bees may result in lower concentrations in other hive food sources. To further refine these estimates, data on the comparative residue levels in flowers, nectar, pollen, and hive products (such as stored pollen, nectar, honey, larval jelly, and beebread) can to be generated to determine worst-case oral exposure estimates for either foraging bees or hive bees.

7.11 HIGHER TIER STUDIES TO ASSESS EXPOSURE OF PESTICIDES TO BEES

7.11.1 Higher Tier Study to Evaluate Contact Exposure to Honey Bees

In the United States, if a compound is classified as toxic to honey bees by contact exposure (i.e., LD50 <11 μg/bee), a Tier 2 contact residue study is required. In this study, a bee attractive plant (typically alfalfa) is sprayed with formulated product at the maximum application rate. Groups of worker bees are caged over the treated crop at various time points after application (typically, 0, 4, 8, and 24 hours), to evaluate the bioavailability and persistence of pesticide residue. These data are used to determine the length of time between application and when bees can be safely exposed to a treated crop. From this test, a residual toxicity time (RT) is established indicating where the pesticide residue is lethal to 25% of the test population, referred to as the RT_{25}.

7.11.2 Higher Tier Exposure Studies Using Honey Bee Colonies

Since it is not economical to conduct exposure studies in every crop, realistic worst-case model crops should be used for assessing exposure of bees under field-relevant use conditions in semi-field and field trials. Choosing a realistic worst-case model crop should include the following considerations:

- attractive to bees
- provides both nectar and pollen
- provides sufficient flower density and sufficient duration of flowering

EPPO PP 1/170 (OEPP/EPPO, 2001) proposes *Phacelia*, oilseed rape (canola), and mustard. Buckwheat (*Fagopyrum esculentum*) may also be used. Application parameters (i.e., rate, interval, formulation) used in any higher tier study should be those that are expected to produce the greatest potential exposure that is prescribed by the product label being assessed.

For a worst-case assessment of exposure, semi-field, or tunnel studies can be conducted. In these studies, colonies are placed within a tent or mesh tunnel and exposed to the treated crop during or immediately after application. Using a highly bee-attractive crop would simulate a worst-case exposure to residues in pollen and nectar. Because of the controlled nature of semi-field studies for foliar-applied products, the location of the study is not as important as it is for a field study. Therefore, data from semi-field studies may be useful in risk assessments beyond the country in which it was performed, assuming that maximum application rates are assessed. However, in some instances, soil type and weather can influence nectar production. See Chapter 8 for additional discussion on effects measurements through semi-field studies.

7.11.3 Studies to Evaluate Exposure from Seed Treatments and Soil Applications of Systemic Compounds

Regarding seed treatments and soil applications with systemic compounds, specific semi-field or field studies can be designed to measure residues in nectar and pollen in order to refine a screening-level risk assessment for systemic compounds. If the purpose of the study is to measure residue data only, the actual crop of interest should be used. If higher tier studies are conducted with a foliar-applied compound and the aim is to concurrently assess residues and potential effects, preferably a crop with the highest application rate and highest attractiveness to bees should be used. If such an effort is undertaken with a systemic compound, then the target crop per se, should be considered first as the test crop, utilizing the maximum application rate for that use scenario. If the target crop is not feasible for conduct of either semi-field or field studies, the use of a surrogate crop is recommended but must be scientifically justified (e.g., supported by plant metabolism data, measured residue levels in nectar and pollen). Data on the uptake and decline of pesticide residues in pollen and nectar after systemic pesticide applications to the test crop should be evaluated prior to initiating field testing with honey bees. (Certain residue chemistry information, typically used for human health assessments may be useful in these cases.) In reviews of reports for two compounds submitted to the State of California (Bireley, 2008; Omer, 2008; Papathakis, 2008; Bireley, 2009), leaf residues in treated perennial shrubs and trees treated with imidacloprid were initially low. Residue levels were below the limit of detection for several weeks after application, but increased to levels above 10 ppm over the next several months in some instances, illustrating that expression of residues in pollen and nectar may follow a curve dependent upon numerous variables. Regardless of the timing of application, it is important that the analysis phase of field studies include sampling of the most important bee-relevant matrices (i.e., pollen, nectar) and characterize the level of residues during plant bloom. Consideration may also need to be given to characterizing the persistence

of residues over time, that is, accumulation from one year to the next (depending upon environmental fate properties).

7.11.4 Field Treatments for Honey Bee Colonies, Spiked Sucrose, and Spiked Pollen

For evaluating the distribution of a pesticide throughout a hive, sucrose, pollen, or protein (pollen substitute) supplements spiked with the proposed test compound (e.g., pesticide active ingredient) should be considered as a potential method of exposure in semi-field and field tests. Spiked pollen, protein (pollen substitute), or sucrose can also be utilized in laboratory and field tests to ensure and accurately quantify exposure to the hive.

When spiked sucrose solution is used as the route of exposure for three or more days, a protein supplement is recommended to ensure that effects observed are due to treatments and not insufficient nutrition. If exposure to the compound is expected to be through pollen collection and feeding, spiked protein can be fed to the test bees. An alternative is to collect and homogenize pollen from a pollen trap, spike the pollen samples with the compound being evaluated, and pressing the spiked pollen into empty combs. However, for some lipophilic compounds, pressing the pollen into a comb could end up extracting the compound if it partitions to the wax. An alternative would be to prepare pollen cake on which the bees can forage. Also, certain pollens should be avoided because they may contain contaminants such as flavonoids that are toxic to bees. In addition, the pollen used should be pesticide free. Finally, the protein content of some pollen, and differences in preference may reduce feeding. In some cases, researchers have used spiked protein supplements. One recommendation is to provide a 500 g protein supplement to the colony each week during a brood cycle (e.g., 21 days). Palatability or toxicity of the test compound may result in the need to alter the size of the supplement. A pollen trap may be used to significantly reduce the quantity of pollen that foraging bees bring into the hive (field studies), thus, encouraging consumption of the spiked protein supplement. A local sucrose feeder may also be used to reduce long distance foraging.

An advantage of using spiked protein supplements is that treated crops are not required and the field size where hives are placed is not relevant as long as there is adequate forage for the number of hives. In these studies, pollen traps can be used to reduce any extraneous pollen from entering the hive. Spiked protein supplements ensure that the hives are exposed to the test substance. Since the protein supplement is not specific to a particular crop, exposure is applicable to any plant where pollen is a food source.

As discussed earlier, appropriate steps should be taken to validate the proper handling of residue samples during collection, shipping, and processing. Validated results indicate that the field handling is appropriate and that the results from the field samples accurately represent actual field residues. See Chapter 8 for more discussion on considerations and conduct of field studies for measuring potential effects.

7.12 HEALTH OF HONEY BEE COLONIES CAN INFLUENCE EXPOSURE

In typically managed colonies, pests and pathogens are present in amounts not necessarily found in the simulated scenarios of laboratory-based or field studies. Honey bee pathogens such as *Nosema* (Fries et al., 2006; Chauzat et al., 2007) and various bee viruses (Chen et al., 2007, 2011; Ribière et al., 2007) are commonly present in managed honey bee colonies. When colonies are subjected to changes caused by pesticide exposure, the pathogen loads can change in honey bees (Alaux et al., 2010; Pettis et al., 2010), and in turn, influence biological and behavioral traits of honey bees. The behavior of diseased honey bees can be modified. For example, diseased honey bees may forage earlier in their life cycle (Ribière et al., 2008), or may be less vigorous foragers, leading to less overall foraging activity and consequently a lower pesticide exposure. Colonies used for testing should be healthy colonies, with minimal levels of pests and pathogens, as these can influence foraging behavior.

7.13 HIGHER TIER STUDIES WITH NON-*APIS* BEE SPECIES

If a screening-level risk assessment does not indicate a presumption of low risk to non-*Apis* bee species, exposure can be evaluated using higher tier studies. In many cases, exposure assessments for honey bee workers may address potential exposure for non-*Apis* bees. However, in some cases, non-*Apis* bees face unique exposure pathways not addressed by exposure assessments for honey bees (see Section 7.2) and consequently, exposure estimates for non-*Apis* bees should be pursued through higher tier studies. Higher tier studies may be pursued solely for exposure information but given their complexity and cost, they likely will be undertaken for information on both exposure and effects. A brief discussion regarding alfalfa leafcutter bees and mason bees provides an example.

7.13.1 Alfalfa Leafcutter Bees: Contamination of Nesting Materials

Alfalfa leafcutter bees (*M. rotundata*) and other species of *Megachile* and *Osmia* will collect leaf pieces from a variety of plants to either wrap or build partitions between their brood cells. Common examples of plants used by these non-*Apis* species include species such as rose (*Rosa* spp.), snow berry (*Symphoricarpos albus*), bindweed (*Convolvulus arvensis*), buckwheat (*F. esculentum*), honeysuckle (*Lonicera* spp.), wild grape (*Vitis vinifera*), or wild senna (*Senna hebecarpa*) (Mader et al., 2010). Alfalfa leafcutter bees deployed for alfalfa pollination also use materials collected from the fields in which they are pollinating and/or foraging. Whether the bees use the target crop or surrounding non-cropped area, there is a potential for exposure from direct application to the crop or drift to adjacent plants.

In the case of the alfalfa leafcutter bee used for alfalfa pollination, it is critical to understand the level of exposure from contaminated leaf pieces and, ultimately, the toxicity of this exposure. See also Chapter 8 on Laboratory Testing Approaches for a discussion on laboratory-based effects studies using treated foliage and see also Chapter 9 for a discussion on considerations with respect to effects information from either semi-field or field studies. One possible approach would be to use a modification of US EPA's guidelines for assessing the toxicity of pesticides on foliage, where alfalfa is sprayed and then brought into a laboratory at various post-application time points, and allowing bees to forage on the foliage. Another approach would be to use a semi-field or field study design as described in the section, Semi-Field Studies.

7.13.1.1 Semi-Field Studies

The following steps relate to assessing potential levels of exposure from contaminated mud, such as with mason bees (e.g., *Osmia cornifrons, Osmia cornuta, Osmia lignaria,* or *Osmia rufa*) that collect mud to build partitions between their brood cells.

1. Plant enclosed shelter (6 m by 2.5 m or larger) with *Phacelia* (*Phacelia tanacetifolia*), sweet clover (*Melilotus* spp.), or other favored forage plant. (Note: In this case, it is also possible to consider the use of artificial nectar or pollen feeder.)
2. Deploy incubated *Osmia* spp. cocoons as loose cells or natal tubes in the enclosure at least 15 days prior to pesticide application (see Bosch and Kemp, 2001; Mader et al., 2010 for management advice).
   - Provided the bees have undergone appropriate diapause (generally 100–200 days at 1.7–4.4°C.), bees will begin emerging 5–10 days after initiating incubation at temperatures of at least 21°C. More rapid emergence can be stimulated by incubating cocoons at 29°C, until all bees have emerged.

- Note that male emergence precedes female emergence, often by several days, and nesting typically will not begin until 1–2 days after mating (which usually occurs on the day of female emergence).

3. Provide a source of wet mud with high clay content in a 1 m wide shallow pan or tray. Water this tray on a daily basis from below in order not to wash pesticide from surface. Ensure that the moisture level is not excessive leading to drowning.
4. Use observation tunnel nests for the bees (i.e., boards with grooves routered into one side (8 mm for *O. cornuta*, 7.5 mm for *O. lignaria*, 6 mm for *O. cornifrons*), covered by a layer of clear acetate and sandwiched with a second piece of wood to create a dark tunnel that can be opened to allow for monitoring.
5. Open observation tunnel nest and note completed cells.
6. Temporarily close tunnel nests and apply the test material to the mud at the levels of interest.
7. Note the new cells created.
8. Open nests and remove the mud partitions that divide the cells in order to measure:
   a. pesticide residue in pollen–nectar stores (pollen ball), and
   b. pesticide residue in mud partitions.
9. Remove exposed cells at 15, 20, and 25+ days to assess the movement of the pesticide into beebread, larval mortality, etc. Depending on the species, full development from egg hatching to adult emergence is completed between 60 and 125 days at 28–17°C. Higher temperatures will result in faster development, but should not exceed 28°C.

Author: Bullet points "(6)Temporarily close nest tunnels and apply pesticide at levels of interest to mud" and "(8)Open nests and pull out mud partitions divided cells provisioned post-application to measure" seems to be unclear. Please check.

7.13.1.2 Field or Semi-Field Studies

1. Deploy leafcutter bees in closable/sealable shelters in an alfalfa field 10 days prior to pesticide application (see Chapter 8 for further discussion on proper incubation timing).
   - Observation tunnel-nests for the bees can be constructed to facilitate monitoring by boring a 0.6 cm ($\frac{1}{4}$ inch) holes or grooves into one side of a wood plank, and covering the holes/grooves with clear acetate. The acetate on such nests should be covered with a removable opaque cover to increase nest attractiveness. The opaque cover can be removed temporarily in order to make notations on the acetate. See also Abbott et al. (2008).
2. During the active nesting period, close the shelter at night to prevent foraging in the green house, cage, or field until the following day. With the nest shelter closed, carefully enter it and note the constructed cells (pre-treatment) in the observation tunnels. With the shelter closed, pesticides can be applied to the field adjacent (at least 200 m radius) around the shelter.
3. After an appropriate time has elapsed (depending upon study goals and active ingredient being used), open the shelter to allow bees to forage, build, and provision the cells.
4. Note new cells created in the observation nests.
5. Newly constructed cells can be monitored for development: eggs will hatch in about 15 days at 15.6°C down to 1–2 days at 35°C. Prior to egg hatching, cells may also be dissected to separate leaf pieces from cell contents (beebread and egg) to assess
   a. pesticide residues in the pollen–nectar mixture (pollen ball), and
   b. pesticide residues on leaf pieces.
6. At 15, 20, and 25+ days, cells can be sampled for the presence of pesticide residues in the pollen ball, monitored for larval mortality, and other parameters. Full development from egg hatching to adult emergence takes 35 days at 15.6°C, but only 11 days at 35°C.

7.13.2 Using Non-*Apis* Bees to Measure Pesticide Contamination of Pollen and Nectar

Using the techniques described here, pollen balls may be removed from the cells of solitary tunnel-nesting bees (e.g., *Osmia* spp. or *M. rotundata*) placed in shelters deployed in fields or orchards treated with pesticides, including systemic pesticides applied as drench or trunk injection. If sufficient forage is available, then these managed non-*Apis* solitary bees typically forage in the area immediately surrounding their nest (40–60 m), thereby helping to ensure that the study organism is coming in contact with the treated plants in well-designed field studies. These bees can also be used readily in semi-field studies as they forage readily in enclosures when provided with adequate forage and nesting material (Bohart and Pedersen, 1963; Abel et al., 2003).

Female foragers of *Osmia* or *Megachile* spp. may also be netted in front of their nest shelters. If they are returning with pollen, it may be gently scraped or brushed from their abdomens or removed by holding the bee with entomological forceps and applying a vibrating tuning fork to the forceps. Note that, unlike honey bees, members of the family Megachilidae, which includes both *Osmia* and *Megachile*, carry pollen in long hairs (scopae) on the underside of their abdomens. This pollen is carried dry, unlike honey bees that carry wet pollen with nectar or honey in order to pack it onto their pollen baskets (corbiculae; Vaissière and Vinson, 1994). It is unknown if wetted pollen may interact with pesticides in the field differently compared to dry pollen.

With regard to nectar contamination, the crop portion of the alimentary track of non-*Apis* bees can be extracted just as easily as with honey bees. Clearly the amount of nectar that can be recovered will be a bit less in smaller species such as mason bees or leafcutter bees, but the procedure is the same as with honey bees. It may be advantageous to anesthetize the foragers prior to squeezing their abdomen gently so as to avoid being stung repeatedly at the same spot though the smaller non-*Apis* species are usually less prone to sting and agile at doing so than honey bees (but this is not true with bumble bee workers).

Field techniques using non-*Apis* bees are presented in greater detail in Chapter 9 on semi-field and field approaches to testing pesticide risk to bees.

7.13.3 Non-*Apis* (Solitary Species) as an Exposure Surrogate for *Apis* Bees

In certain respects, non-*Apis* bees may serve as a useful surrogate for honey bees in exposure studies. Solitary bees, such as leafcutter (*Megachile* spp.) and mason (*Osmia* spp.) bees, typically forage over a much smaller area than honey bees. For example, solitary bees typically forage within a few hundred meters of a nest, rather than two miles (several kilometers) as is common with honey bees. Because of this smaller foraging area, it is possible that a field experiment may provide a more accurate picture of potential exposure, even chronic exposure. Where a honey bee colony will forage over potentially 500 hectares or more, if sufficient forage is present, solitary bees will visit flowers as close to the nests as possible and thus be exposed consistently to local field applications and residues.

7.14 SUMMARY AND RECOMMENDATIONS

Participants of the Workshop agreed that the most significant route of exposure to bees from foliar-applied pesticides is from both contact and oral exposure (of foraging adults, hive adults, and larvae) to contaminated pollen, nectar, and processed food (e.g., beebread, honey, and larval jelly). For systemic compounds (applied as a seed treatment, soil drench, or trunk injection), the most significant route of exposure is through oral ingestion of residues in pollen, nectar, and processed food (e.g., beebread or larval jelly). Other potential routes of exposure include contaminated drinking water and hive material (e.g., contaminated comb wax) and inhalation. For non-*Apis* bee species, unique potential exposure routes include contaminated soil (for solitary ground-nesting species and tunnel-nesting species that use mud to build cell partitions), contact

with sprayed leaves and nesting material that may also be contaminated. Workshop participants agreed that when assessing the major routes of exposure, methods should be conservative enough to account for various potential exposure routes. Unique potential exposure routes, for systemic pesticides, include contaminated abraded dust from seed treatment scenarios, consumption of contaminated aphid honeydew, or possible consumption of contaminated guttation water.

7.14.1 Exposure Estimates

For contact exposure estimates for foliar-applied products, published insect data from direct application exposure studies with honey bees (Koch and Weißer, 1997) can be used to estimate the PEDc of foraging honey bees. Using this data, a worst-case estimate of 1.79 μg/bee is predicted after an application of 1 kg/ha directly to foraging bees.

For non-*Apis* species, Workshop participants recommended using the data for leaf-dwelling and soil-dwelling arthropods from the data developed by Schabacker et al. (2005) to address exposure to leaf-dwelling and soil-nesting non-*Apis* bee species, respectively.

For predicting oral exposure to bees for products applied as spray solutions during crop bloom, there is a limited amount of public data available to make an exposure estimate based on predicted concentrations in pollen and nectar. There is, however, a larger set of proprietary data that may be available from semi-field studies conducted by pesticide registrants. Therefore, Workshop participants discussed the possibility and value of an industry coalition to compile pollen and nectar residue data from both published and proprietary studies to develop a nomogram that can be used to predict concentrations in pollen and nectar based on field application rates. Preferably, a nomogram such as this would contain both mean and 90th percentile predictions.

Pollen and nectar residue levels, reported as mg/kg, can be compared to results from oral exposure toxicity studies with bees if the results of the studies are based on concentrations in the diet, that is, LC50, or as a NOEC (also expressed as mg/kg bee diet). However, if the results from oral exposure toxicity studies are expressed as a median lethal dose (e.g., LD50 in μg/bee), then the predicted exposure dose (in μg/bee) can be calculated based on the concentrations in pollen and nectar, and reported as (adjusted per) consumption rates for different castes of honey bees.

For systemic compounds applied as seed treatment coating, soil applications, or trunk injections, the most significant routes of exposure for adult and larval bees will be through ingestion of pollen, nectar, and processed pollen (i.e., beebread or larval jelly) and processed nectar (i.e., honey). Recognizing the limited field data available to develop exposure models, participants of the Workshop considered the proposal by the ICPBR for a default value of 1 mg/kg in pollen and nectar (Alix and Lewis, 2010), as a potentially appropriate point estimate of exposure for a screening-level assessment for seed treatment and soil applications. Once again, if the results from oral exposure toxicity studies are expressed as a dose (e.g., μg/bee), then the predicted dose can be calculated based on the concentrations in pollen and nectar coupled with reported consumption rates from different castes of honey bees.

7.14.2 Higher Tier Studies to Refine Exposure Assessments

When a screening level assessment indicates potential risks, higher tier studies with applications to bee-attractive plant materials are an option to refine exposure estimates for a specific product. A Tier 2, (contact) toxicity study of residues on foliage with honey bees may be conducted. In this laboratory study a bee-attractive plant (e.g., alfalfa) is sprayed with the formulated product and the bioavailability and persistence of toxic residues are evaluated at various exposure time points after application. The results can be used to

determine the length of time between application and when bees can be safely exposed to residues on leaves or flowers of a treated crop (i.e., RT).

7.14.3 Refining Oral Exposure of Honey Bees to Foliar-Applied Compounds

Tier 3 semi-field or tunnel tests are recommended to refine the oral exposure assessment for honey bee colonies to both systemic and nonsystemic products sprayed on foliage. As discussed in the Hazard–Field section, Workshop participants recommend that semi-field studies should use a bee-attractive crop such as *Phacelia*, oilseed rape (*Brassica napus*), mustard (*Sinapis hirta*), or buckwheat (family Polygonaceae). Use of these study/crop scenarios would provide a better opportunity to ensure exposure because the bees would only have the treated crop to forage on for a specified duration. Therefore, the results from a semi-field test would provide data for a realistic, worst-case prediction of exposure of limited duration resulting from labeled use conditions. In these studies, pollen, nectar, beebread, honey, and if desired, larval jelly can be collected and analyzed for residue levels. Unlike honey bee larvae that consume mostly processed pollen and nectar in the form of brood food and/or larval jelly, many non-*Apis* bee larvae consume only raw pollen. As such, in studies using non-*Apis* bees, oral exposure measurements can be obtained directly via the pollen.

7.14.4 Refining Oral Exposure of Honey Bees to Soil-Applied and Seed Treatment Systemic Compounds

Once again, a semi-field study is recommended for assessing exposure of honey bee colonies to systemic pesticides delivered via seed dressings or through soil treatments. For studies with systemic compounds, the actual crop being assessed should be used, (or potential worst case when multiple crops are being considered) since there may be different rates of uptake, distribution, and metabolism of a compound in different plant species (i.e., between an attractive surrogate crop such as *Phacelia* and a commercial target crop such as melon). Residue analysis should be timed to coincide with the highest nectar/pollen residues expected in the treated crop based on application timing as well as peak residues during bloom. Residues of systemic pesticides in leaves of trees may be highest several months after soil application, indicating that individual characteristics of the treated crop should be considered in assessing the residues in pollen and nectar. Similar to semi-field studies conducted with foliar spray products, residues in pollen, nectar, beebread, honey, and if desired, larval jelly can be collected and analyzed for residues. The measured residue levels can be used in a refined risk assessment.

7.14.5 Refining Exposure of Non-*Apis* Bees

If a screening-level risk assessment indicates potential risk, exposure as well as the effect of a compound to non-*Apis* bee species can be refined using field or semi-field study designs. For assessing exposure to pesticides in pollen and nectar, solitary nesting bees such as blue orchard bees (*O. lignaria*) or alfalfa leafcutter bees (*M. rotundata*), can be used. However, nectar and pollen residue data gained from honey bee trials can also be used to assess exposure for non-*Apis* bees. Similar to studies with honey bees, for foliar-applied pesticides, studies with non-*Apis* bees should be conducted using a bee-attractive crop such as *Phacelia* or sweet clover. Pollen and nectar can be collected directly from the foraging bees. Semi-field or field studies can also be conducted with *Megachile* to evaluate potential (dermal and/or oral) exposure via contaminated nesting material. For assessing exposure to systemic pesticides used as a seed treatment, or applied as a soil treatment or trunk injection, a field study design can be used with these non-*Apis* species to

evaluate worst-case exposure because of the limited foraging range of these species. Potential exposure via soil can also be evaluated using these species.

Author: Please provide citation in the text for the following references: "Van der Steen et al., 2008" "Tapparo et al., 2012" "Kremen et al., 2002" "Cane, 2003" "Chauzat & Faucon, 2007"

REFERENCES

Abbott VA, Nadeau JL, Higo HA, Winston ML. 2008. Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 101(3):784–796.

Abel CA, Wilson RL, Luhman RL. 2003. Pollinating efficacy of *Osmia cornifrons* and *Osmia lignaria* subsp. *lignaria* (Hymenoptera: Megachilidae) on three Brassicaceae species grown under field cages. *J. Entomol. Sci.* 38:545–552.

Alaux C, Brunet J, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* 12(3):774–782.

Alix A, Chauzat MP, Duchard S, Lewis G, Maus C, Miles MJ, Pilling E, Thompson HM, Willner K. 2009a. Guidance for the assessment of risks to bees from the use of plant protection products applied as seed coating and soil applications—conclusions of the ICPBR dedicated working group. *Julius-Kühn Arch.* 423:15–26.

Alix A, Chauzat MP, Duchard S, Lewis G, Maus C, Miles MJ, Pilling E, Thompson HM, Willner K. 2009b. Environmental risk assessment scheme for plant protection products—conclusions of the ICPBR dedicated working group. *Julius-Kühn Arch.* 423:27–33.

Alix A, Vergnet C, Mercier T. 2009c. Risks to bees from dusts emitted at sowing of coated seeds: concerns, risk assessment and risk management. *Julius-Kühn Arch.* 423:131–132.

Alix A, Lewis G. 2010. Guidance for the assessment of risks to bees form the use of plant protection products under the framework of Council Directive 91/414 and Regulation 1107/2009. *EPPO Bull.* 40:196–203.

Atkins EL, Kellum D, Atkins KW. 1981. *Reducing Pesticide Hazards to Honey Bees: Mortality Prediction Techniques and Integrated Management Strategies*. Leaflet 2883. Division of Agricultural Sciences, University of California, Berkeley, CA.

Author: The details in the reference "Bireley, 2008" and "Bireley, 2009" seem to be the same except the year. Please confirm if it is intended so.

Beekman M, Ratnieks FLW. 2000. Long-range foraging by the honey-bee, *Apis mellifera* L. *Funct. Ecol.* 14:490–496.

Bireley R. 2008. Pesticide Evaluation Report Fish and Wildlife Review for Thiamethoxam. Department of Pesticide Regulation, Sacramento, CA.

Bireley R. 2009. Pesticide Evaluation Report Fish and Wildlife Review for Thiamethoxam. Department of Pesticide Regulation, Sacramento, CA.

Bohart GE, Pedersen MW. 1963. The alfalfa leaf-cutting bee *Megachile rotundata* for pollination of alfalfa in cages. *Crop Sci.* 3:183–184.

Bosch J, Kemp W. 2001. *How to Manage the Blue Orchard Bee as an Orchard Pollinator*. Sustainable Agriculture Network, Beltsville, MD, p. 88.

Brewer LW, Sullivan JP, Atkins JM, Kamiri LK, Mihaich EM. 1997. Measured pesticide residues on insects in relation standard EPA estimates. Presented at the 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA.

Author: Please provide the exact date of the meeting for "Brewer et al., 1997."

Cane JH. 2003. Annual displacement of soil in nest tumuli of alkali bees (*Nomia melanderi*) (Hymenoptera: Apiformes: Halictidae) across an agricultural landscape. *J. Kans. Entomol. Soc.* 76:172–176.

Cane J. 2008. Bees (Hymenoptera: Apoidea: Apiformes). *Encyclopedia of Entomology*, Volume 2. Springer Verlag, pp. 419–434.

Cane JH, Griswold T, Parker FD. 2007. Substrates and materials used for nesting by North American *Osmia* bees (Hymenoptera: Apiformes: Megachilidae). *Ann. Entomol. Soc. Am.* 100:350–358.

Author: Please provide the missing editor names in the reference "Cane 2008"

Chauzat MP, Faucon JP. 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera*) in France. *Pest Manag. Sci.* 63:1100–1106.

Chauzat MP, Higes M, Martín-Hernández, Aranzazu Meana R, Nicolas Cougoule N, Faucon, JP. 2007. Presence of *Nosema ceranae* in French honey bee colonies. *J. Apic. Res.* 46:127–128.

Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon JP. 2010. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Aphidae) to monitor pesticide presence in continental France. *Environ. Toxicol. Chem.* 30(1):103–111.

Chen YP, Pettis JS, Collins A, Feldlaufer M. 2007. Prevalence and transmission of honeybee viruses. *Appl. Environ. Microbiol.* 72:606–611.

Chen YP, Evans JD, Pettis JS. 2011. The presence of chronic bee paralysis virus infection in honey bees (*Apis mellifera* L.) in the USA. *J. Apic. Res.* 50(1):85–86.

Choudhary A, Sharma DC. 2008. Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (India). *Environ. Monit. Assess.* 144:143–150.

Dinter A, Brugger KE, Frost NM, Woodward MD. 2009. Chlorantraniliprole (Rynaxypyr): a novel DuponTM insecticide with low toxicity and low risk for honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) providing excellent tools for uses in integrated pest management. *Julius-Kühn Arch.* 423:84–96.

Fischer DL, Bowers LM. 1997. Summary of field measurements of pesticide concentrations in invertebrate prey of birds. Presented at the 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA.

Author: Please provide the exact date of the meeting for "Fischer and Bowers, 1997."

Fletcher JS, Nellessen JE, Pfleeger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for measuring pesticide residues on plants. *Environ. Toxicol. Chem.* 13:1383–1391.

Forster R. 2009. Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008. *Julius-Kühn Arch.* 423:126–131.

Free JB. 1977. *The Social Organization of Honey Bees*. North American Bee Books, Hebden Bridge.

Fries I, Martín R, Meana A, García-Palencia P, Higes M. 2006. Natural infections of *Nosema ceranae* in European honeybees. *J. Apic. Res.* 45:230–233.

Gibbs J, Sheffield CS. 2009. Rapid range expansion of the wool-carder bee, *Anthidium manicatum* (Linnaeus) (Hymenoptera: Megachilidae), in North America. *J. Kans. Entomol. Soc.* 82:21–29.

Girolami VM, Greatti M, Di Bernardo A, Tapparo A, Giorio C, Squartini A, Mazzon L, Mazaro M, Mori N. 2009. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *J. Econ. Entomol.* 102(5):1808–1815.

Goulson D, Stout JC. 2001. Homing ability of the bumblebee, *Bombus terrestris*. *Apidologie* 32:105–112.

Greenleaf SS, Williams NM, Winfree R, Kremen C. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153:589–596.

Hart A, Thompson H. 2001. Estimating pesticide residues on invertebrates eaten by birds and mammals. Poster Presentation at the SETAC 22nd Annual Meeting, Baltimore, MD.

Author: Please provide the exact date of the meeting for "Hart and Thompson, 2001."

Hoeger F, Kenaga E. 1972. Pesticide residues on plants: correlation of representative data as a basis for their estimation of their magnitude in the environment. In: Korte F (ed.), *Environmental Quality and Safety: Chemistry, Toxicology and Technology*. George Thieme Publishers, Stuttgart, pp. 9–25.

Johansen C, Mayer D. 1990. *Pollinator Protection: A Bee and Pesticide Handbook*. Wicwas, Cheshire, CT.

Kim J, Williams N, Kremen C. 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *J. Kans. Entomol. Soc.* 79:309–320.

Koch H, Weißer P. 1997. Exposure of honey bees during pesticide application under field conditions. *Apidologie* 28:439–447.

Konrad RN, Ferry A, Gatehouse, Babenreier D. 2008. Potential effects of oilseed rape expressing oryzacystatin-1 (OC-1) and of purified insecticidal proteins on larvae of the solitary bee *Osmia bicornis*. *PLOS ONE* 3(7):e2664. doi:10/1371/journal.pone.0002664

Kremen C, Williams NM, Thorp RW. 2002. Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl. Acad. Sci.* 99:16812–16816.

Mader E, Spivak M, Evans E. 2010. *Managing Alternative Pollinators*. SARE handbook 11, University of Maryland, College Park, MD, 170 p. http://www.sare.org/publications/pollinators/pollinators.pdf (accessed January 20, 2014).

Malone L, Burgess E, Stefanovic D, Gatehouse H. 2000. Effects of four protease inhibitors on the survival of worker bumblebees, *Bombus terrestris* L. *Apidologie* 31:25–38.

Martel AC, Lair C. 2011. Validation of a highly sensitive method for the determination of neonicotinoid insecticide residues in honeybees by liquid chromatography with electrospray tandem mass spectrometry. *Int. J. Environ. Anal. Chem.* In press.

Author: Please update the reference "Martel & Lair, 2011"

Martel AC, Zeggane S, Aurière C, Drajnudel P, Faucon JP, Aubert M. 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® and Asuntol®. *Apidologie* 38:534–544.

Mason CE. 1986. Progression of knockdown and mortality of honey bees (Hymenoptera: Apidae) sprayed with insecticides mixed with Penncap-M. *Environ. Entomol.* 15:170–176.

Mayer D, Johansen C. 1990. *Pollinator Protection: A Bee and Pesticide Handbook*. Wicwas Press, Cheshire, CT, p. 161.
Michener CD. 2007. *The Bees of the World*, 2nd edn. John Hopkins University Press, Baltimore, MD, p. 913.
Mullin C, Frazier M, Frazier J, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis J. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLOS ONE* 5(3):e9754.
Murphy CM, Breed MD. 2008. Nectar and resin robbing in stingless bees. *Am. Entomol.* 54:37–44.
OEPP/EPPO. 2001. OEPP/EPPO: EPPO Standards PP1/170(3). Test methods for evaluating the side effects of plant protection products on honeybees. *Bull. OEPP /EPPO Bull.* 31:323–330.
Omer A. 2008. Pesticide Evaluation Report Pest and Disease Protection Review for Imidacloprid. Department of Pesticide Regulation, Sacramento, CA.
Orantes Bermejo FL, Gomez Pajuelo A, Megias Megias M, Fernandez Pinar CT. 2010. Pesticide residues in beeswax and beebread samples collected from honey bee colonies in Spain. Possible implications for colony losses. *J. Apic. Res.* 48(1):246–250.
O'Toole C, Raw A. 1999. *Bees of the World*. Blandford, London, p. 192.
Papathakis M. 2008. Pesticide Evaluation Report Chemistry Review for Imidacloprid. Department of Pesticide Regulation, Sacramento, CA.
Pasquet RS, Peltier A, Hufford MB, Oudin E, Saulnier J, Paul L, Knudsen JT, Herren HS, Gepts P. 2008. Long distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proc. Natl. Acad. Sci. U.S.A.* 105:13456–13461.
Pereboom JJM. 2000. The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*. *Insectes Sociaux* 47(1):11–20.
Pettis JS, vanEnglesdorp D, Johnson J, Gively G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften*. 99(2):153–158.
Pistorius J, Bischoff G, Heimbach U, Stähler M. 2009. Bee poisoning incidents in Germany in Spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. *Julius-Kühn Arch.* 423:118–126.
Potts SG, Willmer P. 1998. Compact housing in built-up areas: spatial patterning of nesting aggregations of a ground-nesting bee. *Ecol. Entomol.* 23:427–432.
Pouvreau A. 1984. Biologie et écologie des bourdons. In: Pesson P, Louveaux J (eds), *Pollinisation et Productions Végétales*. INRA, Paris, pp. 595–630.
Ribière M, Ball B, Aubert M. 2008. Natural history and geographical distribution of honey bee viruses. In: Aubert M, Ball B, Fries I, Moritz R, Milani N, Bernadinelli I (eds), *Virology and the Honey Bee*. European Commission, Bruxelles, pp. 15–84.
Ribière M, Lallemand P, Iscache AL, Schurr F, Celle O, Blanchard P, Oliver V, Faucon JP. 2007. Spread of infectious chronic bee paralysis virus by honeybee (*Apis mellifera* L.) feces. *Appl. Environ. Microbiol.* 73(23):7711–7716.
Romaniuk K, Spodniewska A, Kur B. 2003. Residues of chlorinated hydrocarbons in propolis from Warmia and Muzuria voivodship apiaries. *Medycyna Wet.* 11:1023–1026.
Rortais A, Arnold G, Halm MP, Touffet-Briens F. 2005. Modes of exposure of honeybees to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36: 71–83.
Roubik DW. 1989. *Ecology and Natural History of Tropical Bees*. Cambridge University Press, New York, p. 514.
Sampson BJ, Knight PR, Cane JH, Spiers JM. 2007. Foraging behavior, pollinator effectiveness, and management potential of the new world squash bees *Peponapis pruinosa* and *Xenoglossa strenua* (Apidae: Eucerini). *HortScience* 42: 459.
Sapir Y, Shmida A, Ne'eman G. 2005. Pollination of *Oncocyclus irises* (Iris: Iridaceae) by night-sheltering male bees. *Plant Biol.* 7:417–424.
Schabacker J, Barber I, Ebaling M, Edwards P, Riffel M, Welter K, Pascual J, Wolf C. 2005. Review on initial residue levels of pesticides in arthropods sampled in field studies. Report from the European Crop Protection Organization.
Schenke D, Joachimsmeier I, Pistorius J, Heimbach U. 2010. Pesticides in guttation droplets following seed treatment—preliminary results from greenhouse experiments. Presented at the 20th Annual Meeting of SETAC Europe, Seville.
Seiber JN, McChesney MM. 1987. Measurement and computer model simulation of the volatilization flux of molinate and methyl parathion from a flooded rice field. Final Report to the California Department of Food and Agriculture.

Author: Please provide the exact date of the meeting for "Schenke et al., 2010."

Seiber JN, McChesney MM, Majewski MS. 1991. Volatilization rate and downward contamination from application of dacthal herbicide to an onion field. Final Report to the California Department of Food and Agriculture.

Shuler RE, Roulston TH, Farris GE. 2005. Farming practices influence wild pollinator populations on squash and pumpkin. *J. Econ. Entomol.* 98:790–795.

Suchail S, Guez D, Belzunces LP. 1999. Toxicity of imidacloprid and its metabolites in *Apis mellifera*. In: Belzunces LP, Pélissier G, Lewis, GR (eds), *Hazards of Pesticides to Bees*, Vol. 98. INRA, Avignon, pp. 122–126.

Tapparo A, Marton D, Giorio C, Zanella A, Soldà L, Marzaor M, Vivan L, Girolami V. 2012. Assessment of the environmental exposure to honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environ. Sci. Technol.* 46:2592–2599.

Tew JE. 1997. *Protecting honey bees from pesticides*. Factsheet HYG-2161-97. The Ohio State University, Wooster, OH.

Thompson HM. 2001. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus sp.*). *Apidologie* 32:305–321.

Thompson HM. 2007. Assessment of the risk posed to honeybees by systemic pesticides. DEFRA Research Project PS2322.

Thompson HM, Hunt LV. 1999. Extrapolating from honeybees to bumblebees in pesticide risk assessment. *Ecotoxicology* 8:147–166.

Tremolada P, Bernardinelli I, Colombo M, Spreafico M, Vighi M. 2004. Coumaphos distribution in the hive ecosystem: case study for modeling applications. *Ecotoxicology* 13(6):589–601.

Vaissière BE, Merritt SJ, Keim DL. 1985. *Melissodes thelypodii* Cockerell (Hymenoptera: Anthophoridae), an effective pollinator of hybrid cotton on the Texas High Plains. In: Brown JM (ed.), *Proceedings of the Beltwide Cotton Production Research Conference*. National Cotton Council of America, Memphis, TN, pp. 398–399.

Vaissière BE, Vinson SB. 1994. Pollen morphology and its collection effectiveness by honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), with special reference to upland cotton, *Gossypium hirsutum* L. (Malvaceae). *Grana* 33:128–138.

Van der Steen JJM. 1994. Method development for the determination of the contact LD50 of pesticides to bumblebees (*Bombus terrestris* L.). *Apidologie* 25:463–465.

Van der Steen JJM, Bortolloti L, Chauzat MP. 2008. Can pesticide acute toxicity for bumblebees be derived from honeybee LD50 values. Hazards of pesticides to bees—10th International Symposium of the ICPBR Bee Protection Group, October 8–10, Bucharest, Romania.

Vicens N, Bosch J. 2000. Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). *Environ. Entomol.* 29:413–420.

Visscher PK, Seeley TD. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* 63:1790–1801.

Waller G. 1969. Susceptibility of an alfalfa leafcutting bee to residues of insecticides on foliage. *J. Econ. Entomol.* 62:189–192.

Wallner K. 2009. Sprayed and seed dressed pesticides in pollen, nectar and honey of oil seed rape. *Julius-Kühn Arch.* 423:152–153.

Winfree R, Williams NM, Dushoff J, Kremen C. 2007. Native bees provide insurance against ongoing honey bee losses. *Ecol. Lett.* 10:1105–1113.

Winfree R, Williams NM, Gaines H, Ascher JS, Kremen C. 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *J. Appl. Ecol.* 45(3):793–802.

Winston ML. 1987. *The Biology of the Honey Bee*. Harvard University Press. Cambridge, MA.